



United States Department of Agriculture

Research, Education and Economics
Agricultural Research Service

March 18, 2003

Mr. Nathan A. Beaver
McDermott, Will & Emery
600 13th Street, NW
Washington, DC 20005-3096

Dear Mr. Beaver:

This is in further response to your January 15, 2003, Freedom of Information Act (FOIA) request seeking a copy of the testimony drafted by Dr. Paula Cray for the enrofloxacin administrative hearing (FDA Docket 00N-1571).

On February 14, 2003, we provided you with a copy of the draft testimony prepared by Dr. Paula Cray. Before releasing the document to you, a correction was made to the dates on page 2, paragraph 2, which was thought to be an error. However, it has been brought to our attention that the original dates were correct. Therefore, enclosed is a corrected copy of Dr. Cray's testimony. On page 2, paragraph 2, the sentence should read, "From approximately **October** 1998 until May 2000 FSIS conducted the Chicken Monitoring Program for *Campylobacter*."

We apologize for any confusion this may have caused. If you have any questions regarding our response, please contact us at 301-504-1640.

Sincerely,

Valerie Herberger
Freedom of Information Act Coordinator

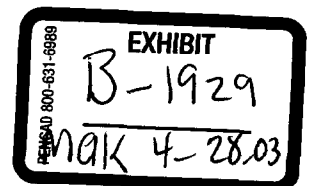
Enclosure



Information Staff, Office of the Director
5601 Sunnyside Ave., Beltsville, MD 20705-5128
Voice: 301-504-1640 □ Fax: 301-504-1647 □ E-mail: vherberger@ars.usda.gov
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2000N-1571

EXH 1



I am Dr. Paula J. Fedorka-Cray. My *curriculum vita* is attached and is Exhibit G- in this record. That Exhibit includes a list of my publications. I earned a Bachelor of Science degree from The Pennsylvania State University in 1979 (Microbiology), a Master of Science degree from North Dakota State University in 1981 (Bacteriology), a MAS from Johns Hopkins University in 1984 (Administration) and a Doctor of Philosophy degree from the University of Nebraska Medical School in 1989 (MSIA - Veterinary Microbiology). I have been employed by the USDA Agricultural Research Service since 1991 and am currently the Research Leader of the Antimicrobial Resistance Research Unit in Athens, Georgia where my research focuses on the ecology of antimicrobial resistance. Additionally, I have served as the Director of the animal arm of the National Antimicrobial Resistance Monitoring System (NARMS) since 1996. Prior to employment by USDA, I was an Assistant Professor (January 1990 - January 1991), an Assistant Instructor (October 1986 - December 1989), and a Research Technologist II (October 1985 - October 1986) in the Department of Veterinary and Biomedical Sciences at the University of Nebraska - Lincoln, NE. From 1981 - 1985, I was employed by the Division of Geographic Medicine at the Johns Hopkins University, Baltimore, Maryland.

Campylobacter isolates from poultry were not added to the animal arm of NARMS until 1998. For the period 1998-2000, all of the *Campylobacter* isolates from poultry in NARMS were obtained from raw product collected by the USDA Food Safety and Inspection Service at federally inspected slaughter and processing establishments throughout the United States and shipped to the ARS Russell Research Center in Athens, Georgia where they underwent antimicrobial susceptibility testing in my laboratory (also see written direct testimony of Geraldine Ransom of FSIS).

FSIS personnel collected rinses from raw poultry carcasses from federally inspected slaughter establishments as described in FSIS Directive 10,230.5. (See written direct testimony of Geraldine Ransom.) The rinses (also called rinsates) were sent to one of three FSIS laboratories (Athens, Georgia; Alameda, California; St. Louis, Missouri) and analyzed for *Campylobacter jejuni/coli* according to the procedures described in the FSIS Microbiology Laboratory Guidebook using the most probable number method. This method is described in detail in the written direct testimony by Geraldine Ransom. In 1998, my laboratory only received isolates from the Athens, Georgia FSIS laboratory. In February 1999, all three FSIS laboratories were directed to submit *Campylobacter* isolates to the ARS laboratory and in

March 1999, ARS began receiving *Campylobacter* isolates from all three FSIS laboratories. Confirmation of *Campylobacter jejuni/coli* was conducted as described in the FSIS Microbiology Laboratory Guidebook and included use of disk diffusion with susceptibility to nalidixic acid and resistance to cephalothin as identification of *Campylobacter jejuni/coli*. Use of nalidixic acid and cephalothin does enable presumptive identification of *Campylobacter jejuni/coli*. It does not enable the differentiation between the species *Campylobacter jejuni* and *Campylobacter coli*. Thus, by use of susceptibility to nalidixic acid as a criteria for selection, isolates would have been expected to be susceptible to nalidixic acid and therefore also susceptible to fluoroquinolones. However, a percentage of the isolates were resistant to nalidixic acid, with additional fluoroquinolone resistance observed for some of the isolates. This suggested that either 'sensitivity' was not absolute as defined by clinical laboratory testing standards or that other phenomenon (described below) were occurring.

From approximately October 1998 until May 2000 FSIS conducted the Chicken Monitoring Program for *Campylobacter*. Rinses from all classes of raw chicken carcasses were analyzed for *Campylobacter jejuni/coli*. The methods used are described in detail in the FSIS testimony by Geraldine Ransom and again used susceptibility to nalidixic acid as diagnostic of *Campylobacter jejuni/coli*.

From November 1999 through November 2000 FSIS conducted the Nationwide Young Chicken (primarily broilers) Microbiological Baseline Data Collection Program to 1) estimate the prevalence of *Salmonella* and 2) for *Campylobacter*, to estimate the prevalence and levels of *Campylobacter jejuni/coli*. Additionally, from January 1999 through October 1999 FSIS conducted the Shakedown Nationwide Young Chicken Microbiological Baseline Data Collection Program for *Campylobacter* to estimate the national prevalence and levels of *Campylobacter jejuni/coli* in young chickens, primarily broilers. (See written direct testimony of Geraldine Ransom.)

The most probable number method described in the FSIS Microbiology Guidebook and used in the FSIS microbiology laboratories for the period from October 1998 through October 2000 for identification of *Campylobacter jejuni/coli* used nalidixic acid susceptibility and cephalothin resistance as part of their identification protocol for identification of *Campylobacter jejuni/coli*. Only isolates

that were nalidixic acid susceptible and cephalothin resistant were considered *Campylobacter jejuni/coli*.

During March 2000 through October 2000 in the Nationwide Young Chicken Microbiological Baseline Data Collection Program for *Campylobacter*, ARS was also sent a group of isolates from FSIS that were considered atypical in that they were nalidixic acid resistant and cephalothin resistant. None of the atypical isolates was included in the NARMS data. Typical isolates from this program as well as the Chicken Monitoring Program were also continuing to be received by ARS during this time period.

In 2001, I assumed responsibility for isolating *Campylobacter jejuni/coli* from the rinses. Culture methodology was changed to protocols routinely used in the ARS laboratory (Englen 2002) and use of nalidixic acid susceptibility and cephalothin resistance as a confirmatory test was discontinued.

Beginning in January 2001, isolation of *Campylobacter* was conducted in my laboratory using spent FSIS *Salmonella* compliance broiler rinsates and using the ARS isolation and identification methods. That method did not use susceptibility to nalidixic acid and resistance to cephalothin for characterization of isolates. For 2001, only rinsates from the Eastern FSIS laboratory (located in Athens, Georgia) were available for antimicrobial susceptibility testing in the animal arm of NARMS. Rinsate from the other FSIS were not considered for use because the process entailed an extra day of shipping, unknown handling practices at the other laboratories, and potential loss of viability from temperature fluctuations.

Also in 2001, two different methods (conventional and spin) for isolation of *Campylobacter* were being used when recovery of *Campylobacter* appeared to be lower for one of the methods. Spent rinsates from the FSIS *Salmonella* compliance program were used. For the "conventional method", upon receipt of the rinsate at ARS, a sterile swab was used to transfer approximately 100ul of the rinsate to 1ml of Bolton Broth for enrichment, followed by plating on *Campylobacter* Cefex agar. A lower recovery (approximately 11%) than previously reported by other laboratories, including reports by FSIS, was observed. Therefore Dr. Cray's laboratory personnel optimized recovery ("spin method") for this type of sample by taking 10 ml of rinsate, centrifuging the rinsate at low speed, decanting the supernatant and enriching the pellet in 10 ml of Bolton Broth prior to plating on *Campylobacter* Cefex agar. No antimicrobials were

used for the identification of *Campylobacter*. However, Cefoperazone (3rd generation cephalosporin), Vancomycin, Trimethoprim Sulfamethoxazole and Cyclohexamide are used in Bolton Broth and Cefoperazone (3rd generation cephalosporin), Nystatin, and Rifampicin are used in the *Campylobacter* Cefex Agar. Currently, we refer to the "spin method" as the "ARS Optimized Method" for isolation of *Campylobacter*.

In 2002, isolation and testing of *Campylobacter* for the animal arm of NARMS is ongoing in the ARS lab using spent FSIS *Salmonella* compliance broiler rinsates and our optimized method, which does not use susceptibility to nalidixic acid and resistance to cephalothin for characterization of isolates. Rinsates are only acquired from the Eastern FSIS laboratory.

Additionally, other phenomenon concerning culture and selection of *Campylobacter* have been observed. *Campylobacter* have the ability to aggregate which confounds recovery of single isolates. This aggregation may be very difficult to overcome as demonstrated by Miller et al., who clearly showed two colony types/species irreversibly aggregating. Further work in our laboratory (Englen, et.al) has demonstrated that serial passage followed by selection of a phenotypic well-isolated colony does not ensure disaggregation. Aggregation of isolates makes both speciation and antimicrobial susceptibility testing nearly impossible as each respective clone within the aggregation may have different antimicrobial resistance patterns and may also be different species. Additionally, MPNs cannot be considered accurate. We estimate that between 6 and 15% of selected colonies result in aggregations although this has not been definitely confirmed on a large scale. Collectively, these data support the difficulty we and others have observed in defining the true prevalence of either sensitive or resistant *Campylobacter* populations. Therefore, while each data set provides accurate information, it is only specific for that set of data which is specific to the culture methodology and selection criteria being used. It is likely that sensitivity to nalidixic acid in a confirmatory step would under-represent total resistance. Further, it is also likely that the Optimized Method, while increasing total numbers, would also increase the recovery of the total number of resistant bacteria.

The following table summarizes this information:

1998 (not a full year)	1999	2000	2001	2002
All classes of chickens (from the FSIS Chicken Monitoring Program)	All classes of chickens (Chicken Monitoring Program)	Broilers (Nationwide Young Chicken Study) and all classes of chickens (Chicken Monitoring Program)	Salmonella program rinsates; Eastern lab only	Salmonella program rinsates; Eastern lab only to date (November)
Nal ^S and Ceph ^K selected isolates	Nal ^S and Ceph ^R selected isolates	Nal ^S and Ceph ^R selected isolates	2 ARS methods compared for part of the year; no Nal ^S and Ceph ^K selection	ARS optimized method; no Nal ^S and Ceph ^K selection